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Prevalence of Antibodies Against Foot-and-Mouth Disease Virus in Cattle in Kasese and Bushenyi Districts in Uganda

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Abstract: The aim of this study was to determine the seroprevalence and serotype-specificity of the circulating antibodies against Foot-and-Mouth Disease Virus (FMDV) in cattle in Kasese and Bushenyi districts in Uganda. A total of 309 serum samples were collected and tested for antibodies against Non-Structural (NS) and Structural Proteins (SP) using Ceditest® FMDV-NS and Ceditest® FMDV type O test kits. Seroprevalences were much higher in Kasese in both tests (61 and 43%, respectively) than in Bushenyi (3 and 4%, respectively). A high proportion of sera, that tested positive in the NSP test, were subjected to seven serotype specific blocking ELISAs for antibodies against the seven FMDV serotypes (O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3). The study showed presence of antibodies against four FMDV serotypes with decreasing magnitude as follows: O> SAT 1> SAT 3/SAT 2. It is recommended to develop sampling schemes to include virus recovery and identification, as well as to focus serum sampling on young unvaccinated stock.

Key words: Antibody, cattle, ELISAs, foot-and-mouth disease, Uganda

INTRODUCTION

Foot-and-Mouth Disease Virus (FMDV) is classified within the *Aphthovirus* genus as a member of the *Picornaviridae* family and is a highly infectious disease agent that causes severe vesicular disease. Foot-and-Mouth Disease (FMD) affects all cloven-hoofed animals including domesticated ruminants and pigs and more than 70 wildlife species (Thomson, 1995). The epidemiology of FMD in Africa was reviewed by Vosloo *et al.* (2002) a decade ago. The salient features of this disease in Africa that were highlighted include; the presence of six FMDV serotypes including serotypes O, A, C, Southern African Territories (SAT) 1, SAT 2 and SAT 3 with only Asia 1 serotype reported negative on the continent. The disease is of high economic importance especially to countries that have an intensive animal industry.

FMD outbreaks occur annually in Uganda's estimated 11.4 million cattle population (Anonymous, 2009), and previous studies have shown incursions of serotypes O, A, SAT 1 and SAT 3 (Vosloo *et al.*, 2002). Efforts to control the disease mainly consist of vaccination and restriction of animal movement in the affected areas. Between 2003 and 2006 FMDV vaccines used have included serotypes O, SAT 1 and SAT 2, and have mainly been imported from Kenya and Botswana. However, these control measures have not stopped the FMD outbreaks, which in 2006 were mostly caused by serotype O (Ayebazibwe *et al.*, 2010), but also with evidence of some SAT outbreaks in 2004 and 2006 (Balinda *et al.*, 2009a; Ayebazibwe *et al.*, 2010).

Several techniques for confirmation of FMDV have been described in the OIE Manual of Diagnostic Techniques (OIE, 2004) but there is still need for considerable effort for developing rapid, accurate tests for use on a wider scale (Clavijo *et al.*, 2004). FMDV can be isolated from cell cultures, or the viral antigen can be detected using ELISAs, while the presence of viral genomic material can be detected using RT-PCR assays. Serological assays for the detection of antibodies against FMDV, irrespective of infection or vaccination status in animals, have been applied in many studies (Berger *et al.*, 1990; Have and Jensen, 1983; Sorensen *et al.*, 1998a), however, these first antibody test systems were serotype-specific, and thus tedious to use for screening in areas where multiple FMDV serotypes are present. Albeit developed with a different scope (Sorensen *et al.*, 2005), the development of serological tests using the FMDV Non-Structural Proteins (NSP), which have shared epitopes between the serotypes, has

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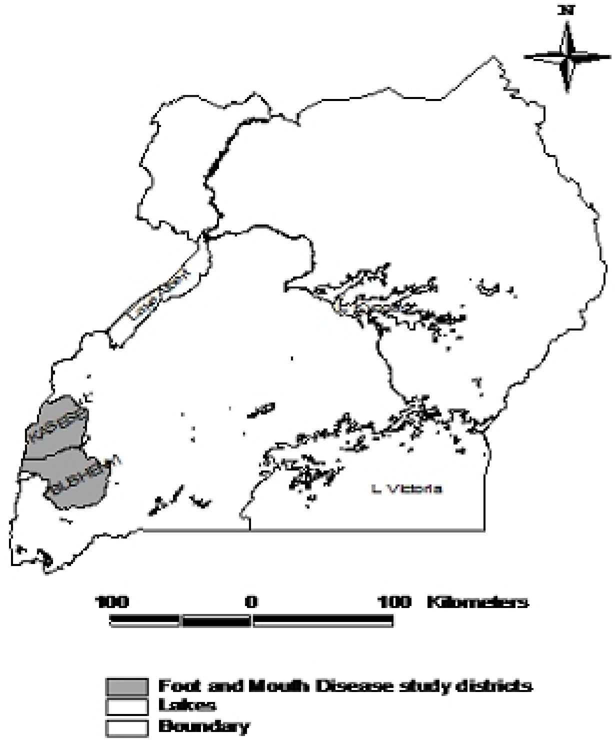

Fig. 1: Map of Uganda showing the districts included in this study

provided a much needed tool for detection of antibodies against FMDV in areas with concurrent activity of more serotypes. Recently, another test system using the structural proteins of serotype O has been developed to detect antibodies against serotypes O (Chénard *et al.*, 2003). This ELISA has been shown to have cross serotype specificity against FMD serotypes A, C and Asia 1, however, the sensitivity of this test for antibodies against serotypes SAT 1, SAT 2 and SAT 3 has, as far as we know, never been evaluated.

The aim of this study was to determine the seroprevalence and serotype-specificity of the circulating antibodies against FMDV in cattle in Kasese and Bushenyi districts in Uganda.

MATERIALS AND METHODS

Study area, sampling, sample collection and handling: This study was carried out between April and June 2007 in two Western Uganda districts: Kasese district, which is often involved in FMD outbreaks and harbors Queen Elizabeth National Park (QENP), and Bushenyi district, which, despite harboring a wildlife reservoir for QENP and bordering this park, has not reported FMD-outbreaks for 10 years, except for a quickly contained outbreak in 2006 (Fig. 1). The farmers in Kasese District predominantly practice communal grazing, while fencing or paddock grazing is mainly practiced in Bushenyi. The counties in these districts were selected for inclusion in the study based on information from the District Veterinary Officers' (DVOs) to the Ministry of Agriculture Animal Industry and Fisheries (MAAIF) on suspected FMD outbreaks. The herds were selected based on consultation with field veterinary officers in the respective districts on investigation of recent FMD outbreaks. The farmers were interviewed about management practice, other animals grazing with cattle, previous exposures to FMDV and vaccination history. With the consent of the farmers, cattle blood samples were taken. Serum was extracted in the field within 24 h of sampling by use of a Mobilespin 12-V field centrifuge (Vulcon Technologies, UK). Aliquots of approximately 4.5 mL of sera were collected, transported on ice and stored at -20ºC at the National Animal Disease Diagnostics and Epidemiology Centre (NADDEC) until needed for serological analysis. A total of 309 cattle sera from 36 herds were collected and analyzed for antibodies against FMDV.

Serological investigation of antibodies against FMDV: All sera were screened for antibodies against FMDV Non-Structural Proteins (NSP) using Ceditest® FMDV-NS kit (Cedi Diagnostics BV, Lelystad, The Netherlands) and against Structural Proteins (SP) of FMDV serotype O

SP-O) using Ceditest® FMDV type O kit (Cedi Diagnostics BV, Lelystad, The Netherlands). Briefly; Ceditest® FMDV-NS kit is a blocking ELISA that detects antibodies against the non-structural 3ABC protein of FMDV of all seven serotypes and it may be used to detect infection of vaccinated animals (Sorensen *et al.*, 2005). Standard protocol procedures were followed according to manufacturer's instructions. Optical Density values (OD) were measured with a Multiskan Ascent spectrophotometer (Thermo Labsystems Oy, UK) using dual wavelengths of 620 nm and 450 nm and Ascent Software, version 2.6. Ceditest® FMDV type O test was also performed according to the manufacturer's instructions. For both kits, the results were expressed as Percentage Inhibition (PI) as follows;

$$PI = 100 - ((OD_{450} - OD_{620})_{\text{test serum}} / (OD_{450} - OD_{620})_{\text{mean negative control}}) \times 100$$

PI <50% was interpreted as negative, while a PI value of ≥50% was positive.

From each herd, 17-100% (average 70%) of sera that tested positive on NSP were selected and screened for antibodies against all the seven FMDV serotypes (O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3) at a fixed dilution of 1/5 using an in-house Solid Phase Blocking ELISA (SPBE) system set up at Lindholm (Balinda *et al.*, 2009b) and implemented at MAAIF. The percentage OD value, ODP, of each individual serum was calculated as the OD value of the test sample as a percentage of the mean OD value of four wells with a negative control serum. The cut off values varied between serotypes. Sera were considered positive, if the ODP was <50% for serotypes O, SAT 1, SAT 2 and SAT 3, <45% for type A, and <35% for serotypes C and Asia 1 (Balinda *et al.*, 2009b). In herds where serotype screening showed reactivity for multiple serotypes in the same herd, representative sera were two-fold diluted from 1/5 to 1/640 for one or more serotypes as appropriate. The antibody titres were calculated as the reciprocal of the last positive dilution in the dilution series.

Statistical analysis: Descriptive statistics were used and frequency distributions calculated (Thrusfield and Bertola, 2005). Prevalences of positive animals were determined by dividing the number of positive serum

Table 1: Prevalences of antibodies against FMDV NSP and FMDV SP-O in cattle from 36 farms in Kasese and Bushenyi districts

District	County	Village	Farm ID	Farm type	No. Sera tested	NSP Positive (%)	SP-O Positive (%)	Last FMD outbreak	Vaccination date
Kasese	Busongora	Kabaka	K1[g]	Communal	24	18 (75)	1 (4)	July 2006	ia
		Busunga	K2[s]	Fenced	9	9 (100)	6 (67)	May and August 2006	ia
			K3	Communal	7	7 (100)	5 (71)	2006	ia
			K4	Fenced	14	13 (93)	12 (86)	2006	ia
			K5[gs]	Fenced	11	7 (64)	2 (18)	1974	2005
		Rwentutu	K6[gs]	Fenced	15	1 (7)	0 (0)	1974	1985
	Bukonjo	Rwembyo	K7[gs]	Fenced	17	6 (35)	1 (6)	2000	October 2006
		Kayanja	K8[g]	Communal	10	6 (60)	5 (50)	April and December 2006	April 2007
			K9[g]	Communal	10	9 (90)	9 (90)	October 2005	April 2007
			K10[g]	Communal	10	6 (60)	5 (50)	ia	ia
			K11[g]	Communal	10	2 (20)	2 (20)	ia	ia
			K12[g]	Communal	10	7 (70)	9 (90)	October 2005	April 2007
	Ibuga		K13	Communal	10	7 (70)	6 (60)	2005	April 2007
	Kisasa		K14[g]	Communal	12	9 (75)	7 (58)	November 2006	April 2007
			K15[g]	Communal	4	3 (75)	3 (75)	ia	ia
			K16[g]	Communal	20	8 (40)	10 (50)	ia	ia
Sub-total					193	118 (61)	83 (43)		
Bushenyi	Bunyaruguru	Mugogo III	B1	Fenced	4	0 (0)	0 (0)	n	nv
		Buzenga II	B2[g]	Fenced	11	1 (9)	0 (0)	1997	nv
		Katara	B3[g]	Fenced	6	0 (0)	0 (0)	1997	nv
	Igara (East)	Nyakahita	B4[g]	Fenced	4	0 (0)	0 (0)	n	May 2006
		Katunda	B5[g]	Fenced	6	1 (17)	0 (0)	n	May 2006
			B6[g]	Fenced	5	0 (0)	0 (0)	n	nv
		Kabushaho	B7[gs]	Fenced	4	0 (0)	0 (0)	n	nv
		Kihunda	B8[g]	Fenced	11	0 (0)	1 (9)	n	March 2007
			B9[s]	Fenced	5	0 (0)	2 (40)	n	March 2007
			B10[gs]	Fenced	3	0 (0)	1 (33)	n	March 2007
	Ruhinda Sheema (North)	Kajwija	B11	Fenced	4	0 (0)	0 (0)	1970	nv
		Kobukyera	B12[g]	Fenced	6	0 (0)	1 (17)	n	March 2007
		Ngoma	B13[g]	Fenced	5	0 (0)	0 (0)	1970	nv
		Mutojo	B14[g]	Fenced	7	0 (0)	0 (0)	n	nv
			B15[g]	Fenced	3	0 (0)	0 (0)	1992	nv
		Kemikyera	B16[g]	Fenced	5	0 (0)	0 (0)	1982	nv
			B17	Fenced	7	0 (0)	0 (0)	n	nv
		Kimondo II	B18[g]	Fenced	6	1 (17)	0 (0)	n	nv
			B19[g]	Fenced	8	0 (0)	0 (0)	n	nv
			B20[g]	Fenced	6	1 (17)	0 (0)	n	nv
Sub-total					116	4 (3)	5 (4)		

[g]: cattle farms with goats only, [s]: cattle farms with sheep only, [gs]: cattle farms with both goats and sheep, ia: information not available, n: never had FMD outbreak, nv: never vaccinated

Table 2: Screening at a dilution of 1:5 of sera from cattle herds in Kasese District for serotype-specific antibodies against FMDV

		Proportion of positive sera per serotype in SPBE 1:5						
Herd ID	No. of sera screened	O	A	C	Asia1	SAT1	SAT2	SAT3
K1^{g}	12	5/12	0/12	0/12	0/12	6/12	10/12	9/12
K2^{s}	9	8/9	2/9	4/9	2/9	7/9	9/9	8/9
K3	7	6/7	0/7	1/7	0/7	6/7	7/7	6/7
K4	13	13/13	1/13	0/13	0/13	5/13	7/13	12/13
K5^{gs}	3	2/3	0/3	1/3	1/3	1/3	2/3	2/3
K6^{gs}	1	1/1	0/1	0/1	0/1	0/1	1/1	1/1
K7^{gs}	5	1/5	0/5	0/5	0/5	2/5	4/5	4/5
K8^{g}	6	6/6	2/6	1/6	0/6	6/6	5/6	6/6
K9^{g}	5	5/5	1/5	1/5	4/5	5/5	5/5	5/5
K10^{g}	1	1/1	0/1	0/1	0/1	1/1	1/1	1/1
K11^{g}	2	2/2	0/2	0/2	1/2	2/2	2/2	2/2
K12^{g}	2	2/2	0/2	0/2	2/2	2/2	1/2	2/2
K13	2	2/2	0/2	0/2	2/2	2/2	1/2	1/2
K14^{g}	4	4/4	0/4	0/4	1/4	4/4	4/4	4/4
K15^{g}	3	3/3	0/3	0/3	0/3	3/3	3/3	2/3
K16^{g}	4	4/4	1/4	0/4	0/4	4/4	3/4	4/4
Total	79	65/79	7/79	8/79	13/79	56/79	65/79	69/79

samples by the total number of samples tested. A herd was considered positive for a given serotype if one or more serum samples had antibody titres ≥160 in the serotype-specific SPBE.

RESULTS

All herds were free from clinical signs of FMD during sampling in 2007. The last outbreak of FMD in these two Districts took place in May-August 2006, 8-11 months before the sampling, and involved at least six of the 16 sampled farms in Kasese district (Table 1), while all of the sampled farms in Bushenyi District had been free from FMD for a prolonged period of time. Vaccination had been carried out 2-6 weeks before the sampling on five Kasese farms (K8, K9, K12, K13 and K14) (Table 1) and in two Bushenyi villages, Kihunda (B8, B9, B10) and Kobukyera (B12).

Prevalence of antibodies against FMDV in Kasese and Bushenyi Districts: Only 4 out of 116 serum samples from Buzenga II (B2), Katunda (B5) and Kimondo II (B18, B20) villages of Bushenyi district were positive for antibodies against NSP, while five other serum samples from Kihunda (B8, B9, B10) and Kobukyera (B12) villages of the same district were found positive for antibodies against SP-O (Table 1).

All sixteen farms in the seven sampled villages of Kasese district, were positive for antibodies against NSP with altogether 61% (118/193) of serum samples testing positive in this test, while only 43% (83/193) were positive for antibodies against SP-O (Table 1). On farm level prevalences for antibodies against NSP were generally high (60-100%), but two farms, one fenced and one practicing communal grazing, had only 7 and 20% of the samples positive.

Seventy-nine of the 122 sera positive for antibodies against NSP were screened at a dilution 1:5 in the SPBE for antibodies against all seven FMDV serotypes (Table 2). Only a few sera reacted positive when screened in the SPBE for antibodies against serotypes A, C and Asia 1 (7, 8 and 13 of the 79 sera, respectively). These sera had higher ODPs for one, or in most cases more, of serotypes O, SAT 1, SAT 2 and SAT 3, and since previous work with this SPBE test system has shown that such reactions are most likely cross-reactions (Ayebazibwe *et al.*, 2010; Balinda *et al.*, 2009b), these reactions were not investigated further.

Table 3: Titres in sera titrated for antibodies against FMDV serotypes O, SAT 1, SAT 2 and SAT 3

Titre*	Number of sera per FMDV serotype			
	O	SAT 1	SAT 2	SAT 3
≤40	19/46	29/54	29/42	36/55
80	12/46	14/54	8/42	8/55
≥160	15/46	11 /54	5 /42	11/ 55

*: Titre expressed as the reciprocal value of the last positive dilution. Cut-off ³160

A high proportion of the 79 tested sera were positive in the SPBEs for antibodies against serotypes O (82%), SAT 1 (71%), SAT 2 (82%) and SAT 3 (87%), and a number of these were titrated in the relevant SPBEs (46/67, 54/58, 42/66 and 55/71, respectively). High antibody titres (≥160) were found in less than one third of the titrated sera for each of serotypes O (33%), SAT 1 (20%), SAT 3 (20%) and SAT 2 (12%) (Table 3), altogether comprising 22 of the titrated sera, of which 12 had this level of antibodies towards two or more serotypes (Table 4).

Animals from the five herds in Kayanja, Ibuga and Kisasa with a recent vaccination history (K8, K9, K12, K13 and K14) generally displayed antibody titres of 80 and above against more than one of the following serotypes; O, SAT 1, SAT 2 and SAT 3, while four herds in Rwentutu, Rwembyo and Busunga had no (K5, K6 and K7, titres ≤ 40) or minimal (K3, one serum with titre 80 against serotype O) evidence of more recent exposure to FMDV.

Table 4: Serotype-specific antibody titres for serotype O, SAT 1, SAT 2 and SAT 3 in sixteen herds in Kasese district

VILLAGE	Farm ID	Field ID	O	SAT1	SAT2	SAT3	Conclusion (s)
Kabaka	K1[g]	18	20	*	80	5	SAT 2 (SAT 1)
		16	nd	5	nd	nd	
		23	*	5	160	20	
		20	*	10	nd	nd	
		19	*	10	nd	*	
		14	nd	80	nd	nd	
		17	nd	*	*	10	
Busunga	K2[s]	28	40	nd	nd	40	O, SAT1, SAT 3
		32	80	5	nd	10	
		31	80	80	nd	80	
		26	160	20	5	160	
		33	160	320	5	160	
		27	320	5	5	80	
		29	nd	40	nd	nd	
		25	nd	*	nd	20	
Busunga	K3	34	40	40	5	40	(O)
		35	80	10	10	40	
		39	*	20	20	10	
		36	nd	40	nd	40	
		38	nd	40	nd	*	
		40	nd	nd	40	5	
Busunga	K4	44	20	20	5	40	O (SAT 1, SAT 3)
		53	40	20	*	20	
		41	40	*	5	10	
		51	80	*	nd	5	
		46	160	40	5	80	
		48	320	*	*	5	
		45	nd	10	*	nd	
		47	nd	80	nd	40	
Busunga	K5[gs]	72	40	*	nd	nd	-
		77	nd	10	nd	nd	
Rwentutu	K6[gs]	62	*	*	5	5	-
Rwembyo	K7[gs]	86	40	20	5	10	-
		84	*	5	5	5	
		88	*	*	*	10	
		94	*	*	5	5	
		97	*	*	5	*	
Kayanja	K8[g]	108	20	10	10	10	SAT 1, SAT 3, O, SAT 2
		100	80	320	80	160	
		103	80	320	80	320	
		101	80	320	*	320	
		107	320	320	160	320	
		99	nd	80	*	40	
Kayanja	K9[g]	113	10	40	20	20	O, SAT1, SAT 3, SAT 2
		118	20	80	20	40	
		109	320	320	320	320	
		110	320	320	5	160	
		114	320	320	160	320	
Kayanja	K10[g]	167	320	160	80	80	O, SAT 1 (SAT2, SAT 3)
Kayanja	K11[g]	182	20	20	40	10	SAT 1, SAT 2 (O)
		176	40	160	40	40	
		175	80	80	160	20	
Kayanja	K12[g]	194	320	80	80	160	O, SAT 1, SAT 3 (SAT2)
		189	320	320	40	80	
Ibuga	K13	121	80	80	40	20	(O, SAT1)
		120	80	80	*	nd	
Kisasa	k14[g]	138	40	40	40	80	(SAT1, SAT 3, SAT 2, O)
		130	40	80	80	80	
		136	40	80	40	80	
		131	80	80	80	80	
Kisasa	K15[g]	143	20	10	5	10	SAT 3 (SAT 2)
		141	40	40	80	320	
		142	40	40	nd	*	
Kisasa	K16[g]	156	80	80	40	40	O (SAT1)
		164	320	10	*	20	
		162	320	10	10	10	
		153	320	80	40	20	

*: negative at screening, nd: not done, positive at screening, but not titrated, most often due to depletion of the sample, [g]: cattle farms with goats only, [s]: cattle farms with sheep only, [gs]: cattle farms with both goats and sheep

High antibody titres against serotype O were recorded in animals in the remaining two herds from Busunga (K2 and K4), one herd from Kayanja (K10) and one herd from Kisasa (K16) with concurrent high (K2 and

K10, titres $\geq$ 160) or borderline (K4 and K16, titres = 80) titres against one or more SAT-serotypes.

High antibody titres against serotype O were absent in the remaining three herds, while there were high titres of antibodies against one or more of the SAT-serotypes (K1, Kabaka: SAT 2; K11, Kayanja: SAT 1 and SAT 2; K15, Kisasa: SAT 3) (Table 4).

DISCUSSION

The two Ugandan Districts investigated in this study had very different status for antibodies against FMDV with much lower seroprevalences of antibodies against FMDV NSP and SP-O in Bushenyi, where only nine of 116 sera were positive in one of the two ELISAs, than in Kasese, where 118 and 83 out of 193 sera were positive for antibodies against NSP and SP-O, respectively.

In Bushenyi, reports of vaccination on farms B8, B9, B10 and B12 some 2-6 weeks prior to the sampling probably accounted for the reactions in the SP-O ELISA, however, the seroprevalences (9-40%) were surprisingly low considering that the trivalent vaccine included serotype O. The reactions seen in the NSP ELISA could be left over antibodies from a rare outbreak in the usually FMD-free Bushenyi District in 2006, or maybe evidence of introduction of animals to this area through trade or traditional exchange, or antibodies elicited by the non-purified vaccines used. These serum samples were not further investigated. A similar study in small ruminants carried out simultaneously on the same farms (Balinda *et al.*, 2009b) also indicated that Bushenyi was free from FMDV in 2007.

In contrast, most Kasese farms had high prevalences of antibodies against FMDV NSP (mean prevalence 61%) as well as against FMDV SP-O (mean prevalence 43%) compared to prevalences of antibodies towards NSP of 14 and 22% in goats and sheep, respectively, reported in the same area by (Balinda *et al.*, 2009b). In cattle, further investigation of the antibodies showed that they were directed mainly towards serotypes O, SAT 1, SAT 2 and SAT 3, while the reactions recorded in the SPBEs for antibodies against serotypes A, C and Asia 1 were of low magnitude and most likely cross reactions of antibodies against other serotypes as has previously been described for this (Ayebazibwe *et al.*, 2010) as well as for another SPCE system (Mackay *et al.*, 2001).

Different serotype profiles were found on different farms. The FMDV-antibody negative serological profiles of one farm in each of Busunga, Rwembyo and Rwentutu villages were consistent with the absence of FMD for a prolonged period of time, and the few cases of titres 40 in two of these herds most likely represented left over antibodies from vaccinations in 2005 and 2006, respectively.

Nine herds reported recent outbreaks of FMD, six in 2006 and three in 2005, and five of these herds in Ibuga, Kayanja and Kisasa reported very recent vaccination, while three herds in Busunga and one in Kabaka did not report vaccination. The serological profiles in the five vaccinated herds accorded with application of the non-purified trivalent vaccine used in Uganda, except for high titres of antibodies against SAT 3, which is not included in this vaccine. One of the four herds that did not report vaccination had an equally mixed serological profile, and it is an open question whether this herd had actually been vaccinated in 2007, while the remaining three farms, one in Kabaka (K1) and two in Busunga (K3 and K4), had serological profiles confirming the lack of vaccination and consistent with older outbreaks of FMDV serotypes SAT 2 and O, respectively.

Of the four herds that neither reported recent FMD outbreaks nor vaccination, two in Kayanja had mixed serological profiles (K10 and K11), and it is not unlikely that these two herds like three other herds in Kayanja had been involved in the 2006 outbreak and had been vaccinated just before the sampling. With regard to the remaining two herds in this group (Kisasa, K15 and K16), serological profiles were more narrow and pointed towards recent exposure to serotypes SAT 3 and O, respectively.

Presence of antibodies against FMDV in cattle was related to presence of antibodies against FMDV in non-vaccinated small ruminants in the same herds in the villages of Busunga, Kabaka, Kayanja and Kisasa, and it was concluded that small ruminants may also be infected during a FMD outbreak (Balinda *et al.*, 2009b). Thus, in addition to the known presence of live FMDV in recovered and persistently infected cattle, these small ruminants may constitute an unrecognized reservoir for FMDV from which the infection could be transferred in case of contact with naïve individuals.

The observed higher seroprevalences and titres of antibodies in cattle than in sheep and goats in the same villages was most likely due to priming of cattle by previous vaccinations with multivalent FMDV vaccines, maybe in combination with lower infection efficacy in small ruminants.

The described serological profiles correlate well with a post-outbreak study of the 2006 FMD outbreak in this area (Ayebazibwe *et al.*, 2010), which showed serological evidence of exposure to FMDV serotypes O and SATs coupled with isolation of FMDV serotype O. This FMD outbreak took place in May-August 2006 and was followed by a vaccination campaign in the area using trivalent non-purified vaccines including FMDV serotypes O, SAT 1 and SAT 2 in October of the same year and in some of the herds in April 2007.

Non-purified vaccines as those used to control FMD in Uganda may elicit antibodies against NSP, especially after repeated use (Sutmoller *et al.*, 2003), and some of the positive reactions in the NSP test presented in this paper are probably due to these vaccinations. Thus, the use of the NSP kit to distinguish infected from vaccinated animals is not reliable in areas like Uganda, where this type of vaccine is used.

Nevertheless, the NSP test has aided serological diagnosis of FMDV as this test can detect antibodies induced by any of the seven serotypes of FMDV (Bronsvoort *et al.*, 2006; Sorensen *et al.*, 1998b). Likewise, the Ceditest FMDV type O kit, which uses purified structural proteins from FMDV serotype O as antigen, has been shown to identify antibodies against FMDV serotypes O, A, C and Asia 1 (Chénard *et al.*, 2003). However, our data indicate that the type O test kit may not be suitable for screening in areas where the SAT-serotypes of FMDV are present. This is discussed in detail by Ayebazibwe *et al.*, (2010).

In this paper, there were concurrent high antibody titres against serotypes O, SAT 1, SAT 2, and SAT 3 in the same serum or herd in allegedly unvaccinated animals (K2, K10, K11, K15 and K16). This was also observed in Ugandan small ruminants (Balinda *et al.*, 2009b) and in a post outbreak study in Ugandan cattle (Ayebazibwe *et al.*, 2010). This reactivity may be due to waning antibodies from previous outbreaks and/or non-reported vaccinations. Lower titres (≤ 80) may represent cross-reactivity between the serotype-specific ELISAs (Balinda *et al.*, 2009b; Mackay *et al.*, 2001). More recent investigations on sera from experimentally vaccinated and infected animals in these ELISAs indicate that especially the SAT 3 antibody ELISA has a high degree of cross-reactivity from antibodies against other serotypes (Tjornehoj *et al.*, Unpublished date). Thus, the high antibody titres against serotype SAT 3 measured in this test, including the one animal from Kisasa (K15), should be interpreted with caution, and can at this point in time not be regarded as conclusive evidence for infection of Ugandan cattle with FMDV serotype SAT 3.

In conclusion, this sampling showed high antibody prevalences in Kasese District, while Bushenyi seemed free from FMDV in 2007. Antibody profiles varied between herds, but reflected the infection and vaccination status at village or herd level. There was serological evidence of past infection with serotypes O, SAT 1 and SAT 2, while evidence of exposure to serotype SAT 3 was not conclusive due to perceived problems with test specificity. It is recommended to develop the procedures for sampling and diagnosis of FMDV to include confirmation of viruses circulating in the area using virus isolation, antigen ELISA and/or RT-PCR and VP1-sequencing. It is also recommended to focus the post outbreak sampling for serological diagnosis on young unvaccinated stock to avoid interference from antibodies elicited by the trivalent non-purified vaccine used in Uganda.

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